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Original Article

Phytochemical Analysis and Antimicrobial Activity of Medicinal Plants Commonly Used in the Treatment of Tuberculosis in the Eastern Free State, South Africa

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ABSTRACT

Background: Tuberculosis (TB), an airborne infectious disease that mainly infects the lungs, caused by *Mycobacterium tuberculosis*. South Africa is one of the countries with the highest TB and multi-drug-resistant TB burden. **Objective:** This study aimed at screening eight medicinal plants that are frequently used by herbalists and traditional healers of the eastern Free State for the treatment of TB. **Methods:** Thirty-two plant extracts were screened for the presence of phytochemicals, antibacterial, antifungal and antimycobacterial activities using standard methods. **Results:** Extracts prepared from *Hermannia depressa* and *Senecio harveianus* displayed the best antibacterial activity against all test microorganisms ranging between 0.098 and 0.781 mg/ml while *Drimia depressa* and *Lotononis lanceolata* extracts displayed the best antifungal activity between 0.049 and 0.781 mg/ml. The good antimycobacterial activity was observed with organic extracts prepared from *Dicoma anomala, H. depressa, L. lanceolata* and *S. harveianus* between 0.195 and 0.781 mg/ml. **Discussion:** All plants displayed the presence of tannins and saponins and the absence of alkaloids, anthraquinones and steroids. Plants under this study demonstrated significant antibacterial, antifungal and antimycobacterial activities, with extracts prepared from *H. depressa* and *S. harveianus* displaying the best activity against all the test microorganisms with MIC values ranging between 0.098 and 0.781 mg/ml, respectively. **Conclusion:** The selected medicinal plants that are used in the treatment of TB and related respiratory ailments in the eastern Free State revealed the presence of phytochemicals and significant antimicrobial activities, which explain and justify their frequent use by traditional healers.

Keywords: Xysmalobium undulatum, Dicoma anomala, Senecio harveianus, Lotononis lanceolata, Drimia depressa, Eucomis autumnalis, Hermannia depressa, Thesium angulosum.

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INTRODUCTION

The use of medicinal plants as a source of therapeutic agents dates back centuries, and their antimicrobial potential has been of significant interest in both traditional and modern medicine. Antimicrobial agents play a vital role in controlling and mitigating the growth of microorganisms, including bacteria and fungi, thereby preventing infections and their associated complications. With the rise of antimicrobial resistance and the limitations of conventional antibiotics, the exploration of natural alternatives, particularly from plant sources, has gained renewed attention. Medicinal plants have been recognized for their diverse chemical composition, which often includes bioactive compounds with inherent antimicrobial properties.

Tuberculosis (TB) is a highly contagious disease caused by a complex *Mycobacterium tuberculosis* strain. The causative agent, *M. tuberculosis*, is transmitted mainly by airborne particles of 1 to 5 µm.[1] The disease mainly infects the lungs (pulmonary TB) but can attack any part in the body, including brain, kidneys, or spine, leading to extrapulmonary TB.[2-3] TB remains a major global health problem and is one of the diseases that causes more deaths than any other infectious disease. The World Health Organization (WHO) reported that TB causes ill-health in millions of people each year and in 2017 was listed among the top 10 causes of deaths worldwide.[4] According to the Global Tuberculosis Report, TB remains the world's top infectious killer with 1.5 million people dying from the disease in 2018.[4]

Globally, approximately 10 million people fall ill with TB every year.[4] There were 10.4 million reported cases of TB infection in 2017 and 1.3 million deaths, despite the availability of anti-TB treatment. Of the reported cases, 90% were adults (5.9 million men and 3.5 million women) and 10% children.[4] Most of the estimated number of cases were found in Asia (61%) and Africa (26%), with India, Indonesia, China, Nigeria, Pakistan, and South Africa accounting for 60% of the global total.[4]

South Africa is one of the countries with the highest TB and multi-drug resistant tuberculosis (MDR-TB) burden. Of the 22 high-burden countries, South Africa has the third highest number of reported cases and the fifth highest number of estimated prevalent cases.[5-6] TB is believed to be one of the major public health problems and the major cause of death among people living with Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome (HIV/ AIDS) than any other disease in the country. The TB bacteria have become resistant to orthodox drugs used to treat TB disease due to the indiscriminate use of anti-TB drugs. According to a report by Churchyard et al. (2014)[7] South Africa has the second highest burden of drug-resistant TB cases in the world. In South Africa, the reported number of

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deaths due to MDR-TB increased by 16.6% between 2013 and 2014.[5,8,9] This development of bacterial resistance to conventional medicine has necessitated the search for new antimicrobial agents.

Medicinal plants were once a primary source of all medicines in the world, and they continue to provide humankind with new remedies.[10] Herbal remedies prepared from medicinal plants have contributed to the reduction of mortality, morbidity and disability caused by cases such as HIV/AIDS, malaria, TB, sickle cell anemia, diabetes, mental disorder, and microbial infections.[11-12] The efforts of researchers in establishing plants with favorable antimicrobial and antimycobacterial properties are yielding prolific results.[13-21] In this study, we assessed the efficacy of seven medicinal plants for their phytochemical constituents, antibacterial activity, antifungal activity, and antimycobacterial activity in treating and managing tuberculosis. The screening of medicinal plants for their secondary metabolites are an important step in drug discovery and natural product research.[22] Secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, often possess bioactive properties that can have therapeutic effects on human health.[23] Further to this study, we have also accessed the antimicrobial and antimycobacterial activities of the medicinal plants against prominent pathogens such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus pumilus* and *Mycobacterium tuberculosis*. *Escherichia coli* and *Klebsiella pneumoniae* are known for causing urinary tract infections and respiratory infections whilst *S. aureus,* and *B. pumilus* are implicated in skin and wound infections.[24-25] *Mycobacterium tuberculosis* presents unique challenges due to its complex structure and the emergence of drug resistance.[26] The study continued to screen medicinal plants for their potential to inhibit the growth and survival of *M. tuberculosis*, shedding light on the pursuit of new therapies against this persistent pathogen. The alarming increase in antibiotic-resistance strains of these pathogens has led to a pressing need for alternative treatment options, spurring interest in exploring medicinal plants as potential sources of antimicrobial and antimycobacterial agents.

MATERIAL AND METHODS

Study sites

The Free State, an inland province in South Africa, is located within the latitudes 26.6° and 30.7° S and longitudes 24.3° and 29.8° E.[27] It is an area with flat, rolling grassland and crop fields rising to sandstone mountains in the northeast. The province comprises five municipal districts: Motheo, Xhariep, Lejweleputswa, Fezile Dabi and Thabo Mofutsanyana. According to a news report by Seleka, (2022)[28] thousands of people are suffering from TB in the Free State province although TB is a manageable disease in South Africa. During the 2010 period, the Free State province reported 24 420 cases of all TB types with Motheo district registered 7 948 cases, followed by Lejweleputswa district with 6 683 cases, Thabo Mofutsanyana with 4 748 cases, Fezile Dabi with 3 601 cases and Xhariep which registered 1 441 patients.[28] According to the Free State Provincial Strategic Plan,[29] HIV and TB are the leading causes of death in the province, with TB being the second most leading cause.

Plant collection

Plant species (Table 1) were collected with the assistance of traditional healers from Thabo Mofutsanyana District municipality. The voucher specimens of each plant were prepared and deposited in the herbarium at the University of the Free State, QwaQwa campus. The voucher specimen numbers were HLON01 for *Xysmalobium undulatum*, HLON02 for *Dicoma anomala* (Sond.), HLON03 for *Senecio harveianus*, HLON04 for *Lotononis lanceolata*, HLON05 *Drimia depressa*, HLON6 for *Eucomis autumnalis* and HLON07 for *Thesium angulosum*.

Plant extraction

The collected plant material was oven dried for 50°C for 24 hours before being ground to a fine powder using a laboratory blender. The powdered plant material was extracted using the standard ratio of 1 g to10 ml of the extraction solvent. Four separate samples of 30 g of powdered plant material were extracted with 300 ml methanol, ethanol, acetone and water. Extraction was done by shaking the mixture for 24 hours at room temperature. Plant extracts were filtered through Whatman No.1 filter paper discs and air dried in a stream of air. This was done by continually weighing plant extracts until a constant weight was obtained. Various extract yields were given by different plant extracts during extraction. Dried plant extracts were stored in sealed bottles at approximately 4°C until further use.

Phytochemical screening

Phytochemicals such as alkaloids, anthraquinones, flavonoids, saponins, steroids and tannins were determined using standard procedures as described by Harbone, (1973)[30] Trease and Evans, (1989)[31] and Sofowora, (1996)[32] whereas cardiac glycosides and terpenoids were detected using the killer-Killani and Salkowski tests, respectively.[33]

Antibacterial activity

Escherichia coli (ATCC 8739), *Klebsiella pneumoniae* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538) and *Bacillus pumilus* (ATCC 10702) were used. A microplate method of Eloff, (1998)[34] was used to determine the minimal inhibitory concentration (MIC) values for plant extracts. Dried plant extracts were redissolved at 50 mg/ml with their extracting solvents. All extracts were initially tested at 12.5 mg/ml and serially diluted to 0.098 mg/ml and tested in triplicate. An antibiotic neomycin was used as a standard in each assay, while extract-free solution was used as a blank control. The microplates were incubated overnight at 37 °C. As an indicator of bacterial growth, 40 µl p-iodonitrotetrazolium violet (INT) dissolved in water at 0.2 mg/ml

Table 1. Medicinal plants are commonly used in the treatment and management of TB in the eastern Free State.

Family name	Scientific name	Common name	Part Used	Uses
Apocynaceae	*Xysmalobium undulatum (L.)* W.T.Aiton	*Poho-tsehla*	Whole plant	Colic, diarrhea, dysentery, hysteria and tuberculosis
Asteraceae	*Dicoma anomala* (Sond.)	*Hloenya*	Leaves	Stomach pains, tuberculosis, wounds, blood pressure and cold
Asteraceae	*Senecio harveianus* MacOwan	*Kgotodiya*	Whole plant	Fever, tuberculosis, cold, coughs and chronic diseases
Fabaceae	*Lotononis lanceolata* (E.Mey.) Benth.	*Kgonathi*	Tuber	Fever, contagious diseases and tuberculosis
Hyacinthacea	*Drimia depressa* (Baker) Jessop	*Moretele*	Bulb	Stomach pains, fever and common cold, headache, tuberculosis and coughs
Hyacinthacea	*Eucomis autumnalis* (Mill.) Chitt.	*Mathithibala*	Bulb	Stomach pains, enema for lower backache, piles, gonorrhea, tuberculosis, coughs and common cold
Sterculiaceae	*Hermannia depressa* N.E. Br.	*Seletjane/ Phate yangaka*	Roots	Heartburn, antidote for food poisoning, tuberculosis and used as a charm for witchcraft
Santalaceae	*Thesium angulosum* DC.	*Lentsweni*	Whole plant	Fever, headache, period pains, coughs and tuberculosis

was added to the wells and incubated at 37 °C for 30 min. MIC values were recorded as the lowest concentration of the extract that completely inhibited bacterial growth, i.e., a clear well.

Antifungal activity

Standard strains of *Candida albicans* (ATCC 10231) and *Trichophyton mucoides* (ATCC 10231) were used. A broth microdilution test was performed to evaluate the antifungal activity of plant extracts.[35-36] Four milliliters of sterile saline were added to approximately 400 µl of 24-h-old fungal cultures. The absorbance was read at 530 nm and adjusted with sterile saline to match that of a 0.5 McFarland standard solution. From the prepared stock cultures, a 1:1000 dilution with broth (e.g., 10 µl stock fungal culture: 10 ml broth) was prepared. The water extract residues were dissolved in water whereas organic solvent extracts were dissolved in dimethyl sulfoxide (DMSO). All extracts were dissolved to a concentration of 100 mg/ml. The aqueous extracts were initially tested at 25 mg/ml while organic solvent extracts were tested at 6.25 mg/ml. Three replicates were prepared for each extract. Amphotericin B (1.5 mg/ml) was used as a standard for this experiment. The microplates were incubated overnight at 33°C. As an indicator of fungal growth, 50 µl of INT solution (0.2 mg/ml) was added to the wells and incubated at 33°C for 30 min.

Antimycobacterial activity

Mycobacterium tuberculosis (ATCC 25177) was maintained in Middlebrook 7H9 broth containing 10% OADC (oleic acid + albumin + dextrose + catalase). The inoculum was prepared by transferring the stock mycobacterium culture to the supplemented 7H9 (Middlebrook 7H9 + 10% OADC) and grown for 72 hours at 37 °C. The broth microdilution method was used to determine the MIC values of plant extracts against *M. tuberculosis*[37]. The residues of aqueous extract were dissolved in water, while those of organic solvent extracts were dissolved in DMSO. All extracts were dissolved to a concentration of 100 mg/ml. All extracts were tested at a concentration of 25 mg/ml and serially diluted to 0.195 mg/ml. The optical density of the 72-h broth culture was determined and adjusted at 550 nm. One hundred microlitres of the diluted culture were added to every well of the microtitre plate. The controls included the solvent used to dissolve plant extracts, Middlebrook 7H9 broth alone and the antibiotic streptomycin (1.56 mg/ml) as a positive control. The plates were covered and incubated at 37°C for 72 h. After incubation, 40 µl of 0.4 mg/ml solution of INT was added to each well of the plate. The plates were covered and incubated for 24 h at 37°C. All extracts were tested in triplicate.

RESULTS

Phytochemical analysis

Eight medicinal plant species belonging to 6 plant families were screened for the presence of secondary metabolites and the results of their phytochemical analysis and chemical composition are presented in Table 2 and Table 3. All 8 plant species tested positive for the presence of saponins whilst *H. depressa* was the only plant species that tested positive for the presence of flavonoids. Steroids, anthraquinones, and alkaloids were not found in any of the plant species that were investigated. From the eight plant species investigated for the presence of secondary metabolites, only *D. anomala*, *D. depressa*, *H. depressa*, *L. lanceolata*, *T. angulosum* and *S. harveianus* tested positive for tannins. Only the *D. anomala*, *D. depressa* and *T. angulosum* tested positive for the presence of terpenoids whilst cardiac glycoside was only present in *D. anomala*, *H. depressa* and *T. angulosum*.

Antibacterial activity

A total of 32 extracts prepared from eight medicinal plants used by the people of the eastern Free State for the treatment and management of tuberculosis were examined for their antibacterial potential against four reference strains of human pathogenic bacteria (*K. pneumoniae*, *B. pumilus*, *E. coli*, *S. aureus*). The sensitive serial dilution microplate method was used to determine the minimum inhibitory concentration of plant extracts against test bacterial strains. The antibacterial activity of specific concentrations of aqueous, acetone, ethanolic, and methanolic extract of different plant parts are presented in Table 4. Plant extracts with MIC values ranging between 0.098 and 0.781 mg/mL were considered highly active, those with MIC values ranging between 1.56 and 3.125 mg/mL were considered moderately active, and poor antibacterial activity when the MIC value was ≥6.25 mg/mL. The recorded MIC values demonstrated that most plant extracts prepared from both organic and aqueous solvents were theoretically effective in suppressing bacterial growth. The acetone and ethanolic extracts prepared from *D. anomala* possessed varying antibacterial activity against the four test pathogenic bacteria with MIC values ranging from 0.098 to 0.781 mg/mL. Moderate antibacterial activity was observed with the aqueous and methanol extracts of *D. anomala* with MIC values of 3.125 mg/mL. A high antibacterial activity was observed with *D. depressa* water extract at 0.098 mg/mL. The ethanolic and methanolic extracts of *D. depressa* displayed moderate antibacterial activity with MIC values ranging between 1.563 and 3.125 mg/mL against test pathogenic bacteria. Poor antibacterial activity (MIC values ranging between 6.250 and 12.5 mg/mL) was observed with *D. depressa* acetone extract. Good activity was observed with *E. autumnalis* acetone and water extracts at 0.098 and 0.195 mg/mL, respectively, while the ethanolic and methanolic extracts displayed moderate antibacterial activity with MIC values of 1.563 and 3.125 mg/mL, respectively. Very good antibacterial activity was observed with all extracts prepared from *H. depressa* and *S. harveianus* against all test microorganisms with MIC values ranging between 0.098 and 0.391 mg/mL. The extracts prepared from *L. lanceolata* showed good to moderate inhibitory activity against all the screened bacterial strains with inhibitory concentration values ranging between 0.098 mg/mL and 0.781 mg/mL. Poor antibacterial

Table 2: Phytochemicals observed from the medicinal plant species used against TB in the eastern Free State.

Plant name	Part used	Phytochemicals							
		Tannin	Saponin	Flavonoid	Steroid	Terpenoid	Cardiac glycoside	Anthraquinone	Alkaloids
D. anomala	Leaves	+	+	+	-	+	+	-	-
D. depressa	Bulb	+	+	-	-	+	-	-	-
E. autumnalis	Bulb	-	+	-	-	-	-	-	-
H. depressa	Roots	+	+	+	-	-	+	-	-
L. lanceolata	Tuber	+	+	-	-	-	+	-	-
T. angulosum	Whole plant	+	+	-	-	+	-	-	-
S. harveianus	Whole plant	+	+	-	-	-	+	-	-
X. undulatum	Whole plant	-	+	-	-	-	-	-	-

- : absent, + : present

Table 3: Chemical compositions of the plants used against TB in the eastern Free State.

Plant name	Part used	Chemical Composition	References
D. anomala	Whole plant	Chemical characterisation- acetylenic compounds, diterpene, flavonoids, phenolic acids, phytosterols, saponins, sesquiterpene lactones, tannins and triterpenes were found. The sesquiterpene lactones included germacranolides, with a 7,8-lactone function, albicolide and 14-acetoxydicomanolide, with 6,7-lactone were also isolated and identified.	38-43
D. depressa	Bulb	Chemical reported- bufadienolides, 3b,16b-dihydroxy-5b-bufa-20,22-dienolide, 16b-hydroxy-5b-bufa-20,22-dienolide-3b-O-b-D-galactoside, hellibrigenin-b-D-glucoside and hellibrigenol-b-D-glucoside.	44
E. autumnalis	leaves and bulbs	Chemicals identified- L-ascorbic acid, carotenoids, and polyphenols ((3-benzyl-4-chromanone, benzylidene and scillascillin type). In total, 16 types of phytochemicals were also identified in *Eucomis autumnalis* subspecies *autumnalis*. These consisted of *eucomic* acid, derivatives of hydroxybenzoic and hydroxycinnamic acids, as well as flavonoids.	45
H. depressa	Bulb	Compounds identified- Lauric acid, Myristic acid, Palmitic acid, Stearyl alcohol. Tannins, saponins, phenolics were also identified.	46
L. lanceolata	Whole plant	Compounds identified- 38 different pyrrolizidine alkaloids, and non-proteic aminoacids, mainly in its seeds	47
T. angulosum	Whole	32 compounds such as flavanones, flavones, flavonols and phenylpropanoids isolated thus far from the aboveground and belowground parts. Apigenin, kaempferol, luteolin, quercetin and isorhamnetin derivatives,	48
S. harveianus	Whole plant	Not found	
X. undulatum	Roots	Chemical identified- cardenolide cardiac glycosides, uzarin (5.6%) and xysmalorin (1.5%), and the isomers allouzarin (0.4%) and alloxysmalorin (0.1%). The cardenolide aglycones, uzari genin and xysmalogenin are present as minor constituents, together with allouzarigenin, alloxysmalogenin, ascleposide, coroglaucigenin, corogluaucigenin-3-O-glucoside, pachygenol, pachygenol-3β-O-glucoside, desglu-couzarin, smalogenin, desglucoxysmalorin, uzaroside,pregneno-lone and β-sitosterol.	49

Table 4. Antibacterial activity of medicinal plants used against TB in the eastern Free State (MIC values in mg/mL).

Plant name	Plant part used	Extract solvent	Bacterial species			
			K. pneumoniae	B. pumilus	E. coli	S. aureus
D. anomala	Leaves	Acetone	0.781	0.098	0.195	0.098
		Ethanol	0.781	0.781	0.098	0.391
		Methanol	3.125	3.125	3.125	3.125
		Water	3.125	3.125	3.125	3.125
D. depressa	Bulb	Acetone	12.5	12.5	6.250	12.5
		Ethanol	1.563	1.563	1.563	1.563
		Methanol	3.125	3.125	3.125	3.125
		Water	0.098	0.098	0.098	0.098
E. autumnalis	Bulb	Acetone	0.391	0.195	0.195	0.098
		Ethanol	3.125	3.125	1.563	1.563
		Methanol	3.125	3.125	3.125	3.125
		Water	0.195	0.195	0.195	0.195
H. depressa	Roots	Acetone	0.098	0.098	0.098	0.098
		Ethanol	0.098	0.098	0.098	0.098
		Methanol	0.195	0.098	0.098	0.098
		Water	0.391	0.391	0.098	0.098
L. lanceolata	Tuber	Acetone	0.195	0.195	0.391	0.098
		Ethanol	1.563	1.563	1.563	1.563
		Methanol	1.563	0.195	1.563	0.098
		Water	6.250	3.125	0.098	0.098
T. angulosum	Whole plant	Acetone	0.098	0.098	0.098	0.098
		Ethanol	1.563	1.563	0.098	1.563
		Methanol	6.250	6.250	6.250	6.250
		Water	6.250	3.125	0.781	0.391
S. harveianus	Whole plant	Acetone	0.391	0.098	0.098	0.098
		Ethanol	0.391	0.391	0.391	0.391
		Methanol	0.195	0.195	0.098	0.391
		Water	0.781	0.781	0.098	0.391
X. undulatum	Whole plant	Acetone	6.250	6.250	6.250	6.250
		Ethanol	1.563	1.563	1.563	1.563
		Methanol	6.250	6.250	0.098	6.250
		Water	3.125	3.125	3.125	3.125
Neomycin (µg/mL)			0.391	0.781	0.781	0.781

Table 5. Antifungal activity of medicinal plants used against TB in the eastern Free State (MIC in mg/mL).

Plant name	Plant part	Extracts	Fungal species	
			C. albicans	T. mucoides
D. anomala	Leaves	Acetone	0.391	0.195
		Ethanol	0.391	0.195
		Methanol	0.781	0.781
		Water	1.563	3.125
D. depressa	Bulb	Acetone	0.098	0.195
		Ethanol	0.098	0.195
		Methanol	0.049	0.195
		Water	0.391	0.781
E. autumnalis	Bulb	Acetone	0.049	0.049
		Ethanol	0.049	0.049
		Methanol	3.125	0.049
		Water	1.563	3.125
H. depressa	Roots	Acetone	0.391	1.563
		Ethanol	0.391	0.391
		Methanol	0.391	0.391
		Water	1.563	1.563
L. lanceolata	Tuber	Acetone	0.049	0.049
		Ethanol	0.391	0.049
		Methanol	0.391	0.049
		Water	1.563	0.781
T. angulosum	Whole plant	Acetone	0.391	3.125
		Ethanol	0.391	1.563
		Methanol	0.391	1.563
		Water	0.781	0.781
S. harveianus	Whole plant	Acetone	1.563	1.563
		Ethanol	1.563	1.563
		Methanol	3.125	0.049
		Water	6.25	12.5
X. undulatum	Whole plant	Acetone	0.781	0.391
		Ethanol	0.781	0.391
		Methanol	1.563	3.125
		Water	3.125	1.563
Amphotericin B (µg/mL)			0.012	0.012

Table 6. Antimycobacterial activity of medicinal plants used against TB in the eastern Free State (MIC in mg/ml).

Plant name	Plant part	Extracts	M. tuberculosis
D. anomala	Leaves	Acetone	0.195
		Ethanol	0.195
		Water	6.25
D. depressa	Bulb	Acetone	6.25
		Ethanol	12.5
		Methanol	12.5
		Water	12.5
E. autumnalis	Bulb	Water	3.125
H. depressa	Roots	Acetone	0.781
		Ethanol	0.781
		Methanol	0.781
		Water	1.563
L. lanceolata	Bulb	Acetone	0.195
		Ethanol	0.195
		Methanol	0.781
T. angulosum	Roots	Acetone	0.391
S. harveianus	Whole plant	Acetone	0.195
		Ethanol	0.195
		Methanol	0.195
		Water	0.391
X. undulatum	Whole plant	Methanol	6.25
Streptomycin (µg/ml)			0.012

activity was observed with *T. angulosum* methanolic extract at 6.250 mg/mL, whereas the acetone extract displayed very good activity against all the test bacterial strains at 0.098 mg/mL.

Antifungal activity

Table 5 presents the antifungal activity results of extracts prepared from eight medicinal plants under investigation. Plant extracts with MIC values ranging between 0.098 mg/mL and 0.781 mg/mL were considered highly active, those with MIC values ranging between 1.56 mg/mL and 3.125 mg/mL were considered moderately active, and those with MIC values ≥6.25 mg/mL were considered to possess poor antifungal activity. Overall, the extract of all the tested plant species showed strong activity and/or demonstrated some degree of activity against the test fungal species. Very good activity was observed with the organic solvents extracts of *D. anomala* against *C. albicans* and *T. mucoides* with MIC values ranging between 0.195 and 0.781 mg/mL, whereas moderate activity was observed with the water extract against the same test fungal strains at 1.563 and 3.125 mg/mL. Very good antifungal activity was observed with extracts prepared from *D. depressa* against the test fungal strains with MIC values ranging between 0.098 and 0.391 mg/mL. Moderate antifungal activity was observed with *E. autumnalis* water extract against *C. albicans* and *T. mucoides* at 1.563 and 3.125 mg/mL, respectively. Very good activity (at MIC values of 0.391 mg/mL) was observed with organic solvents extracts prepared from *H. depressa* and *T. angulosum* against both fungal species. Good antifungal activity was observed with *L. lanceolata* extracts between 0.049 and 0.781 mg/mL, while the water extract displayed moderate activity against *C. albicans* at 1.563 mg/mL. Poor antifungal activity was observed with the water extract prepared from *S. harveianus* with MIC values of 6.25 and 12.5 mg/mL.

Antimycobacterial activity

The results for antimycobacterial activity of plant extracts against *M. tuberculosis* are presented in Table 6. Plant extracts were considered to be highly active if their MIC values ranged between 0.098 mg/mL and 0.781 mg/mL, moderately active if their MIC values ranged between 1.56 mg/mL and 3.125 mg/mL, and extracts with MIC values ≥6.25 mg/mL were considered to possess poor antimycobacterial activity. The acetone and ethanolic extracts prepared from *D. anomala*, acetone, ethanolic and methanolic extracts of *H. depressa*, *L. lanceolata*, *T. angulosum* and *S. harveianus* were found to possess good antimycobacterial activity against *M. tuberculosis* with MIC values ranging between 0.195 to 0.781 mg/mL. Poor antimycobacterial activity was observed with *D. depressa* extracts and *X. undulatum* methanolic extract with MIC values ≥6.25 mg/mL.

DISCUSSION

The presence of saponins was observed in all screened plants. Saponins were reported to possess an array of bioactivities including antimicrobial, antifungal, anti-oxidant, antimutagenic, cytotoxicity, anti-inflammatory and immunostimulatory.[50] The presence of tannins was observed in all screened plants except *E. autumnalis* and *X. undulatum*. According to a study by Francis et al. (2002)[51] plants rich in tannins possess astringent, hemostatic, antiseptic and toning properties. Flavonoids were only observed in *H. depressa* whilst terpenoids were present in *D. anomala, D. depressa* and *T. angulosum*. Terpenoids and tannins are known to possess analgesic and anti-inflammatory activities.[52] Flavonoids are reported to possess antioxidant, antibacterial, anti-inflammatory, analgesic and anti-allergic properties.[53-54] According to a number of studies, phytochemicals generally serve as natural antibiotics that help the body fight infections and invasion by pathogenic mircoorganisms.[52,55,56] Several studies have isolated and identified compounds from the plants under the study, except for *S. harveianus*.

Extracts prepared from *H. depressa* and *S. harveianus* displayed the best activity against all the test microorganisms with MIC values ranging between 0.098 and 0.781 mg/ml. A study conducted by Appidi et al. (2009)[57] also reported on the antibacterial activity of *Hermannia* species against the test bacterial strains. According to Reid, (2002)[58] *H. depressa* extracts exhibited the highest activity, which may play a role in combating coughs, diarrhea and stomachaches caused by bacterial infections. To the best of my knowledge, there is no available report in literature on the antibacterial activity of *S. harveianus*. The acetone and ethanol extracts of *D. anomala* also exhibited the best antibacterial activity with MICs ranging between 0.098 and 0.781 mg/ml. Studies undertaken by Reid et al. (2005)[46] and Mabona et al. (2013)[59] also demonstrated noteworthy antibacterial activity of the organic extracts. The best activity was also observed with *D. depressa* and *E. autumnalis* water extracts at 0.098 and 0.195 mg/ml, respectively. The aqueous extract prepared from *L. lanceolata* displayed the best antibacterial activity against *E. coli* and *S. aureus* with MIC values of 0.098 mg/ml. *L. lanceolata* acetone extract also displayed the best activity with MIC values ranging between 0.098 and 0.391 mg/ml. Moderate activity was observed with ethanolic extract. To the best of my knowledge. there are no reported studies on the biological activity of *L. lanceolata* in literature. *T. angulosum* acetone extract displayed the best activity against all test bacterial strains at 0.098 mg/ml, whereas water extract displayed good activity against *E. coli* and *S. aureus* at 0.781 and 0.391 mg/ml, respectively.

The organic solvents extracts displayed the best activity against *C. albicans* and *T. mucoides* compared to water extracts. The organic extracts prepared from *D. anomala, E. autumnalis, H. depressa, L. lanceolata* and *X. undulatum* demonstrated the best activity between 0.049 and 0.781 mg/ml. Extracts prepared from *D. depressa* displayed the best activity where they inhibited both fungal strains between 0.049 and 0.781 mg/ml. Concerning water extracts, the best antifungal activity was detected with *D. depressa*, *T. angulosum* and *L. lanceolata*. In a study undertaken by Eloff, (1998)[60] acetone was reported as the best extractant for the screening of antimicrobial components in plants, followed by methanol: chloroform: water, methylene dichloride, methanol, ethanol and water. Moderate or poor activity with water might be due to that aqueous solvents do not extract all the active compounds that might be present in the plants .[62] Hence, the traditional medication is applied at high volumes.[36]

The organic extracts prepared from *D. anomala, H. depressa, L. lanceolata* and *S. harveianus* displayed the best antimycobacterial activity with MIC values ranging between 0.195 and 0.781 mg/ml. Good activity was observed with *H. depressa* and *S. harveianus* water extracts at 1.561 and 0.391 mg/ml, respectively. *Drimia depressa, E. autumnalis* and *X. undulatum* displayed weak activity against *M. tuberculosis*. According to Green et al. (2010)[15] weak activity in vitro does not imply plant extracts would demonstrate weak activity in vivo. Some plants may be more potent in vivo due to metabolic transformation of their components into highly active intermediates.[15,62] Poor or weak activity might be due to the physical barrier provided by the complex lipoglycan calyx on the cell surface.[63] It is believed that lack of penetration is the reason why many antibiotics become resistance to *M. tuberculosis*.[26]

CONCLUSION

The scientific investigation of the potential antibacterial, antifungal and antimycobacterial activities of plants used by the traditional healers and herbalists in treating TB is important. The selected medicinal plants used against TB and other respiratory ailments in the Free State Province demonstrated significant antibacterial, antifungal and antimycobacterial activities which may explain and justify the usage of the plants by traditional healers and herbalists. In addition, most of the plant species revealed the presence of phytochemicals such as

tannins, saponins, steroids, cardiac glycosides and terpenes, with one of the plant species having flavonoids. Work is being undertaken to screen plant extracts for anti-inflammatory activity and cytotoxicity and to isolate active compounds from the plants that displayed good antimicrobial activity.

AUTHORS CONTRIBUTIONS

M.H. conducted the experiments, analyzed results and drafted the manuscript; S.L. critical revision of the article for important intellectual content and final approval of the article; L.B.K. supervised the experiments, edited and finalized the manuscript

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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